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- A rhodamine dye or a salt thereof, comprising a rhodamine-type parent xanthene ring having attached to the xanthene C9 carbon a phenyl group that is further substituted with an ortho carboxy or ortho sulfonate group or a salt thereof, one to three substituted or unsubstituted aminopyridinium groups and a substituted or unsubstituted alkylthio, arylthio or heteroarylthio group, said rhodamine dye optionally including one or more linking moieties.
  - 2. The rhodamine dye of Claim 1 which comprises the structure:

$$\begin{pmatrix} R \\ N \end{pmatrix} \qquad \qquad \begin{pmatrix} Y \\ N \end{pmatrix} \qquad \qquad \begin{pmatrix} COOH \\ S-Z \end{pmatrix}$$

wherein:

n is 1, 2, or 3;

Y is a rhodamine-type parent xanthene ring attached to the illustrated phenyl group at the xanthene C9 carbon;

each R is independently selected from the group consisting of  $(C_1-C_6)$  alkyl and heteroalkyl,  $(C_5-C_{20})$  aryl and heteroaryl,  $(C_6-C_{26})$  arylalkyl and heteroalkyl,  $(C_5-C_{20})$  arylaryl and heteroaryl-heteroaryl, or when taken together, R is  $(C_4-C_{10})$  alkyldiyl,  $(C_4-C_{10})$  alkyleno,

20 heteroalkyldiyl and heteroalkyleno;

S is sulfur:

Z is  $(C_1-C_{12})$  alkyl,  $(C_1-C_{12})$  alkyl substituted with one or more of the same or different W<sup>1</sup> groups,  $(C_5-C_{20})$  aryl and heteroaryl, and  $(C_5-C_{20})$  aryl and heteroaryl substituted with one or more of the same or different W<sup>2</sup> groups;

W¹ is selected from the group consisting of -X, -R,  $\neq O$ , -OR, -SR, =S, -NRR, =NR,  $-CX_3$ , -CN, -OCN, -SCN, -NCO, -NCS, -NO,  $-NO_2$ ,  $=N_2$ ,  $-N_3$ ,  $-S(O)_2O^{-}$ ,  $-S(O)_2OH$ ,  $-S(O)_2R$ , -C(O)R, -C(O)X, -C(S)R, -C(S)X, -C(O)OR,  $-C(O)O^{-}$ , -C(S)OR, -C(O)SR, -C(O)NRR, -C(O)NRR, -C(O)NRR, and -C(O)NRR;

 $W^2$  is selected from the group consisting of -R, -OR, -SR, -NRR,  $-S(O)_2O^2$ ,

 $-S(O)_2OH, -S(O)_2R, -C(O)R, -C(O)X, -C(S)R, -C(S)X, -C(O)OR, -C(O)O^*, -C(S)OR, -C(O)O^*$ -C(O)SR, -C(S)SR, -C(O)NRR, -C(S)NRR and -C(NR)NRR;

each X is independently a halogen; and

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3. The rhodamine dye of Claim 2 in which L is selected from a hydrophobic moiety, a charged group, a member of a pair of specific binding molecules, a photo-activatable group and a reactive functional group.

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The rhodamine dye of Claim 2 where Z has the form  $Z^1$ -L-R<sub>x</sub>, or a salt thereof, 4. wherein:

 $Z^1$  is  $(C_1-C_{12})$  alkyldiyl,  $(C_1-C_{12})$  alkyldiyl independently substituted with one or more of the same or different W1 groups, or

(C<sub>5</sub>-C<sub>14</sub>) aryldiyl, and aryldiyl, heteroaryldiyl and heteroaryldiyl independently substituted with one or more of the same or different W<sup>2</sup> groups;

L is a bond or a linker; and

 $R_x$  is a reactive functional group.

5. The rhodamine dye of Claim\4 in which Y is selected from:

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(Y-1)

(Y-2)

;

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$$(Y-3) \qquad R^{15} \qquad R^{6'} \qquad R^{5} \qquad R^{4} \qquad R^{3} \qquad R^{16} \qquad R^{17} \qquad R^{17$$

; and

$$(Y-4) \qquad R^{15} \stackrel{R^{6'}}{\stackrel{}{\stackrel{}{\stackrel{}{\stackrel{}{\stackrel{}{\stackrel{}}{\stackrel{}{\stackrel{}}{\stackrel{}}{\stackrel{}{\stackrel{}{\stackrel{}}{\stackrel{$$

and a salt thereof, wherein;

R<sup>1</sup> and R<sup>2</sup> when taken alone, are independently hydrogen or (C<sub>1</sub>-C<sub>6</sub>) alkyl;

 $R^3$  and  $R^3$  when taken alone, are independently selected from the group consisting of hydrogen,  $(C_1-C_6)$  alkyl,  $(C_5-C_{14})$  are and arylaryl, or when taken together is  $(C_4-C_6)$  alkyldiyl or alkyleno, or when individually taken together with  $R^2$  or  $R^4$  is  $(C_2-C_6)$  alkyldiyl or  $(C_2-C_6)$  alkyleno;

 $R^4$ , when taken alone, is selected from the group consisting of hydrogen and  $(C_1-C_6)$  alkyl, or when taken together with  $R \setminus C_2-C_6$  alkyldiyl or alkyleno;

 $R^5$ , when taken alone, is selected from the group consisting of hydrogen and  $(C_1-C_6)$  alkyl, or when taken together with  $R^6$  or  $R^6$  is  $(C_2-C_6)$  alkyldiyl or alkyleno;

 $R^6$  and  $R^6$  when taken alone, are selected from the group consisting of hydrogen,  $(C_1-C_6)$  alkyl,  $(C_5-C_{14})$  aryl and arylaryl, or when taken together are  $(C_4-C_6)$  alkyldiyl or alkyleno, or when individually taken together with  $R^5$  or  $R^7$  is  $(C_2-C_6)$  alkyldiyl or alkyleno;

R<sup>7</sup>, when taken alone, is selected from the group consisting of hydrogen and  $(C_1-C_6)$  alkyl, or when taken together with R<sup>6</sup> or R<sup>6'</sup> is  $(C_2-C_6)$  alkyldiyl or alkyleno;

 $R^8$ , when taken alone, is selected from the group consisting of hydrogen and  $(C_1-C_6)$  alkyl;

R<sup>9</sup> indicates the point of attachment to the *ortho*-carboxyphenyl bottom ring; and R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup>, R<sup>13</sup>, R<sup>14</sup>, R<sup>15</sup>, R<sup>16</sup>, R<sup>17</sup>, R<sup>18</sup>, R<sup>19</sup>, R<sup>20</sup> and R<sup>21</sup> are each independently selected from the group consisting of hydrogen and (C<sub>1</sub>-C<sub>6</sub>) alkyl, or

6. The rhodamine dye of Claim 5 wherein R<sup>2</sup>, when taken together with R<sup>3</sup> or R<sup>3</sup> is (C<sub>2</sub>-C<sub>6</sub>) alkyldiyl or alkyleno.

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7. The rhodamine dye of Claim 6 wherein: alkyl is methanyl, ethanyl or propanyl; aryl is phenyl or naphthyl;

arylaryl is biphenyl;

alkyldiyl or alkyleno bridges formed by taking R<sup>2</sup> together with R<sup>3</sup> or R<sup>3</sup>, R<sup>7</sup> together with R<sup>6</sup> or R<sup>6</sup>, or R<sup>4</sup> together with and R<sup>3</sup> or R<sup>3</sup>, are ethano, propano, 1,1-dimethylethano, 1,1-dimethylpropano and 1,1,3-trimethylpropano;

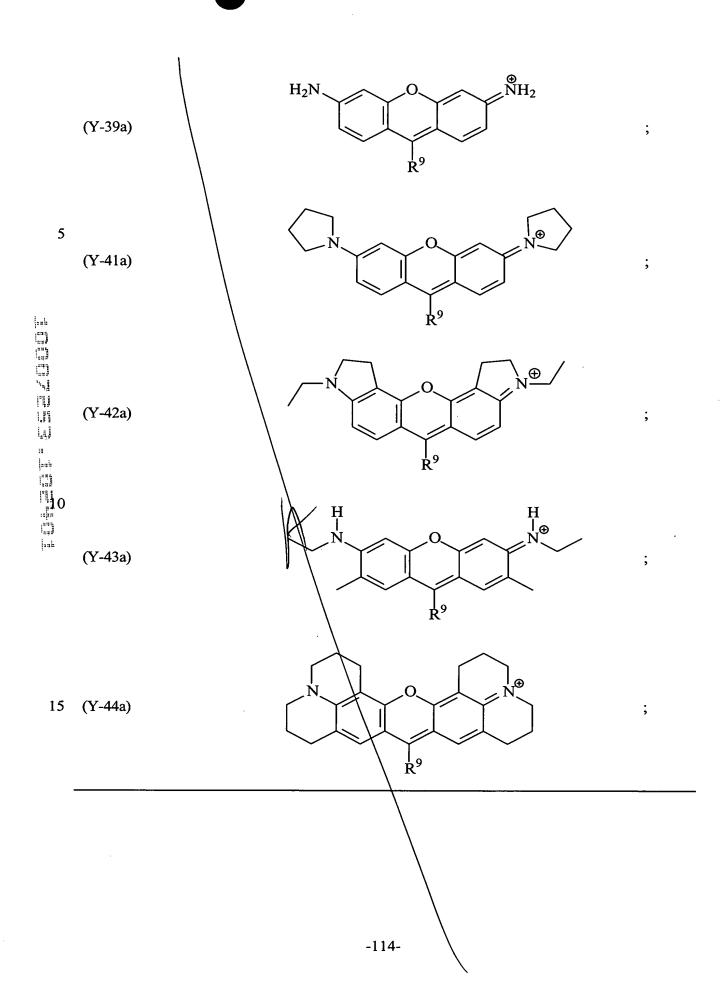
aryleno bridges formed by taking R<sup>1</sup> together with R<sup>2</sup> are benzo or naphtho; alkyldiyl or alkyleno bridge formed by taking R<sup>3</sup> together with R<sup>3</sup>, or R<sup>6</sup> together with R<sup>6</sup>, is butano;

alkyldiyl or alkyleno bridges formed by taking R<sup>5</sup> together with R<sup>6</sup> or R<sup>6'</sup> are ethano, 20 propano, 1,1-dimethylethano, 1,1-dimethylpropano and 1,1,3-trimethylpropano; and aryleno bridge formed by taking R<sup>10</sup>, R<sup>11</sup>, R<sup>12</sup> and R<sup>13</sup> together, or R<sup>18</sup>, R<sup>19</sup>, R<sup>20</sup> and R<sup>21</sup> together, is benzo.

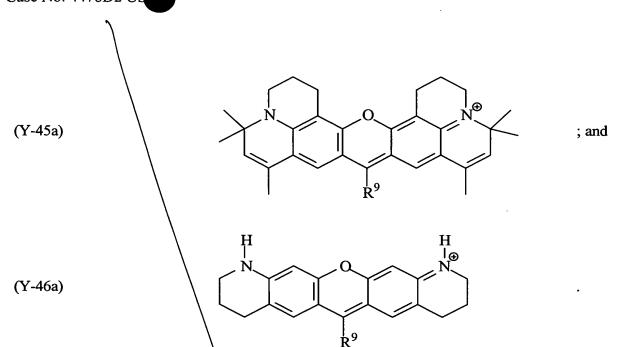
8. The rhodamine dye of Claim 6 in which L is a bond.

- 9. The rhodamine dye of Claim 4 in which  $R_{\chi}$  is selected from the group consisting of carboxyl, carboxylate, ester and activated ester.
- 10. The rhodamine dye of Claim 4 in which Z<sup>1</sup> is selected from the group consisting of 30 (C<sub>1</sub>-C<sub>12</sub>) alkyleno, (C<sub>1</sub>-C<sub>12</sub>) alkano, (C<sub>5</sub>-C<sub>10</sub>) aryldiyl and heteroaryldiyl, phenyldiyl, phena-1,4-diyl, naphthadiyl, naphtha-2,6-diyl, pyridindiyl and purindiyl.

The rhodamine dye of Claim 4 in which Y is selected from the group consisting of: 11. 5 (Y-20a) To the state of th (Y-21a) (Y-22a)  $H_2N$ (Y-23a) 15 (Y-24a)



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- 12. The rhodamine dye of Claim 4 in which L is a bond.
- 13. The rhodamine dye of Claim 4 in which  $R_{\chi}$  is selected from the group consisting of carboxyl, carboxylate, ester and activated ester.
- 14. The rhodamine dye of Claim in which Z<sup>1</sup> is selected from the group consisting of 15 (C<sub>1</sub>-C<sub>12</sub>) alkyleno, (C<sub>1</sub>-C<sub>12</sub>) alkano, (C<sub>5</sub>-C<sub>10</sub>) aryldiyl and heteroaryldiyl, phenyldiyl, phena-1,4-diyl, naphthadiyl, naphtha-2,6-diyl, pyridindiyl and purindiyl.
  - 15. The rhodamine dye of Claim 4 which comprises the structure:

or a salt thereof.

- 16. The rhodamine dye of Claim 15 in which Y is selected from the group consisting of Y-1, Y-2, Y-3 and Y-4.
- 17. The rhodamine dye of Claim 15 in which Y is selected from the group consisting of Y-20a, Y-21a, Y-22a, Y-23a, Y-24a, Y-35a, Y-36a, Y-36a, Y-39a, Y-41a, Y-42a, Y-43a, Y-44a, Y-45a and Y-46a.
  - 18. The rhodamine dye of Claim 2 which has the structure:

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$$\begin{pmatrix} R \\ V \end{pmatrix} \begin{pmatrix} R \\ V \end{pmatrix} \end{pmatrix} \begin{pmatrix} R \\ V \end{pmatrix} \begin{pmatrix} R \\ V \end{pmatrix} \begin{pmatrix} R \\ V \end{pmatrix} \end{pmatrix} \begin{pmatrix} R \\ V \end{pmatrix}$$

wherein:

Y<sup>1</sup> is a rhodamine-type parent xanthene ring attached to the illustrated phenyl group at the xanthene C9 carbon;

L is a bond or linker attached to a xanthene nitrogen atom or a xanthene C4 carbon;

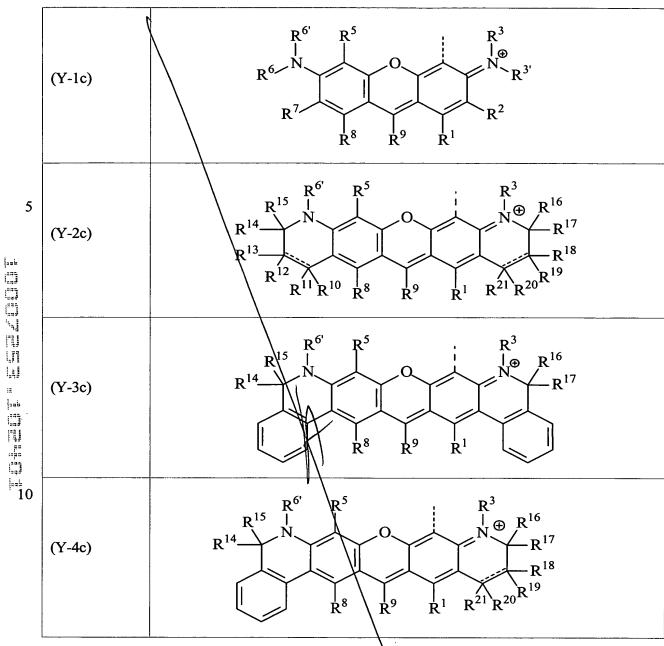
n is 1, 2, or 3; and

 $R_x$  is a reactive functional group.

19. The rhodarnine dye of Claim 18 in which Y<sup>1</sup> is selected from the group consisting

of:

10	(Y-1b)	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$
The state of the s	(Y-2b)	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$
15	(Y-3b)	$R^{14} \xrightarrow{R^{15}} \stackrel{R^{6'}}{{}{}{}{}{}{{$
20	(Y-4b)	$R^{14}$ $R^{15}$ $R^{16}$ $R^{16}$ $R^{17}$ $R^{18}$ $R^{18}$ $R^{18}$ $R^{18}$ $R^{19}$



wherein the dashed line at the nitrogen or C4 atom indicates the point of attachment of substituent L.

20. The rhodamine dye of Claim 19 wherein: alkyl is methanyl, ethanyl or propanyl; aryl is phenyl or naphthyl;

arylaryl is biphenyl;

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alkyldiyl or alkyleno bridges formed by taking R<sup>2</sup> together with R<sup>3</sup>, R<sup>4</sup> together

with R<sup>3</sup>, R<sup>5</sup> together with R<sup>6</sup>, or R<sup>7</sup> together with R<sup>6</sup>, are ethano, propano, 1,1-dimethylpropano and 1,1,3-trimethylpropano;

aryleno bridges formed by taking  $R^{10}$ ,  $R^{11}$ ,  $R^{12}$  and  $R^{13}$  together or  $R^{18}$ ,  $R^{19}$ ,  $R^{20}$  and  $R^{21}$  together are benzo.

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21. The rhodamine dye of Claim 18 in which L is selected from the group consisting of  $(C_1-C_6)$  alkyldiyl,  $(C_1-C_6)$  alkano,  $(C_5-C_{20})$  aryldiyl, phenyldiyl, phena-1,4-diyl, naphthyldiyl, naphtha-2,6-diyl, naphtha-2,7-diyl,  $(C_6-C_{26})$  arylalkyldiyl  $-(CH_2)_i-\phi$  and  $-(CH_2)_i-\psi$ , where each i is independently an integer from 1 to 6,  $\phi$  is  $(C_5-C_{20})$  aryldiyl, phenyldiyl or phena-1,4-diyl and  $\psi$  is naphthyldiyl, naphtha-2,6-diyl or naphtha-2,7-diyl.

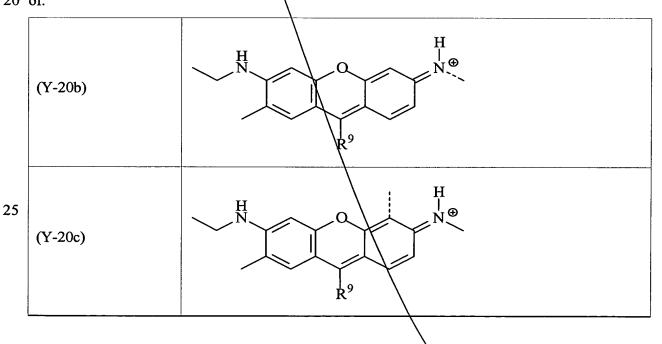
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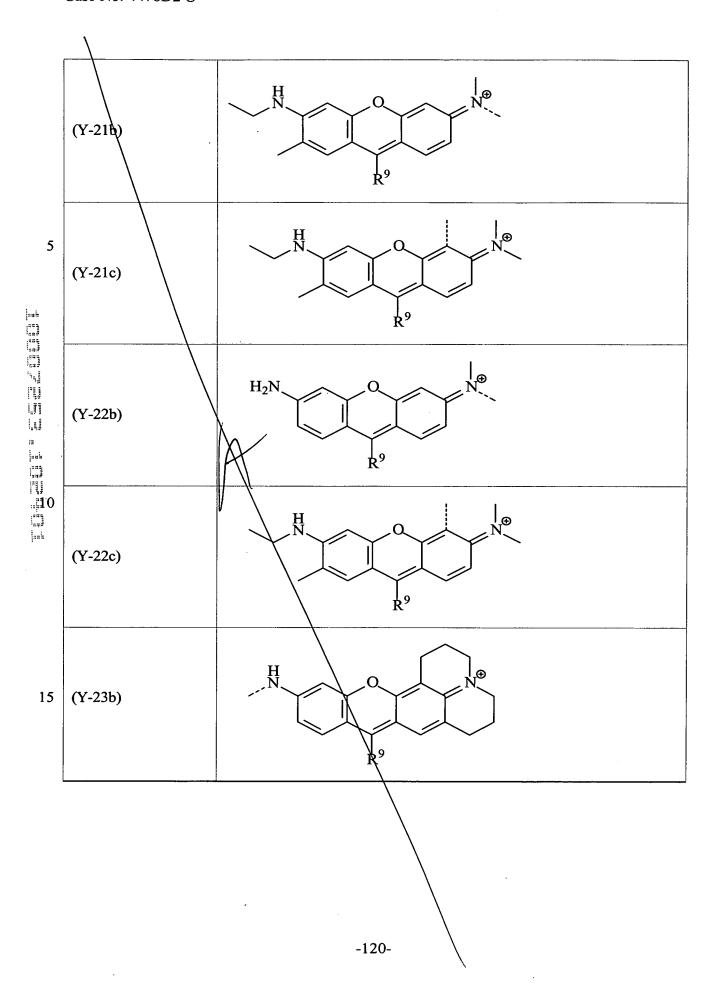
22. The rhodamine dye of Claim 18 in which  $R_x$  is selected from the group consisting of carboxyl, carboxylate, ester and activated ester.

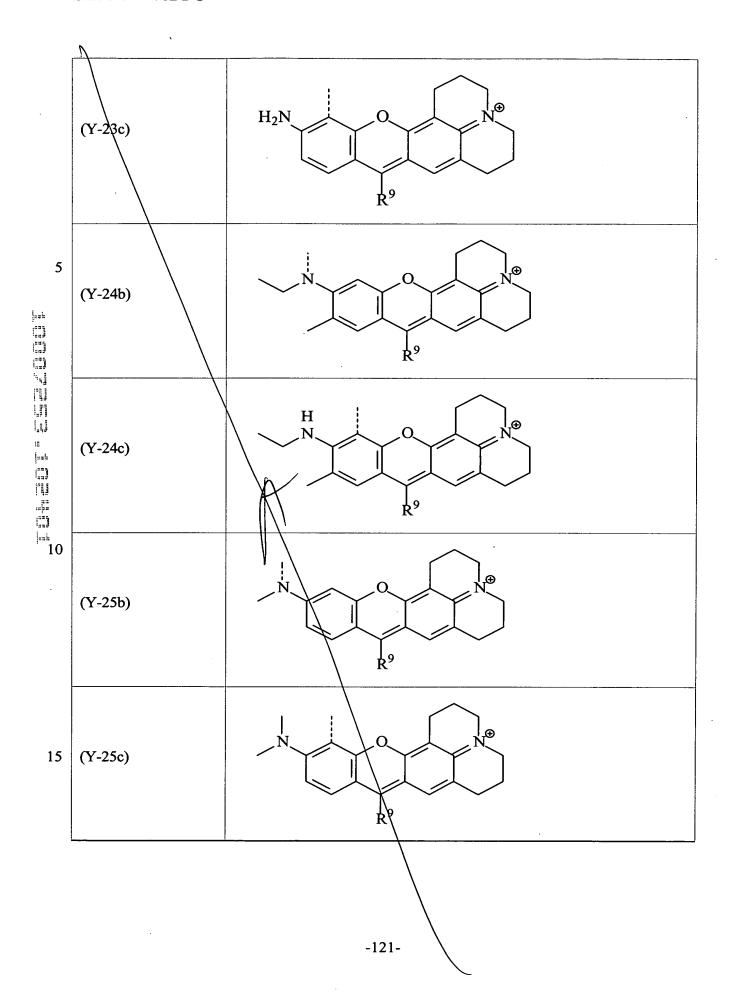
23. The rhodamine doe of Claim 18 in which Z is selected from the group consisting of  $(C_1-C_{12})$  alkyl,  $(C_1-C_{12})$  alkanyl,  $(C_3-C_{10})$  aryl and heteroaryl, phenyl, naphthyl, naphth-1-yl, naphth-2-yl, pyridyl and purinyl.

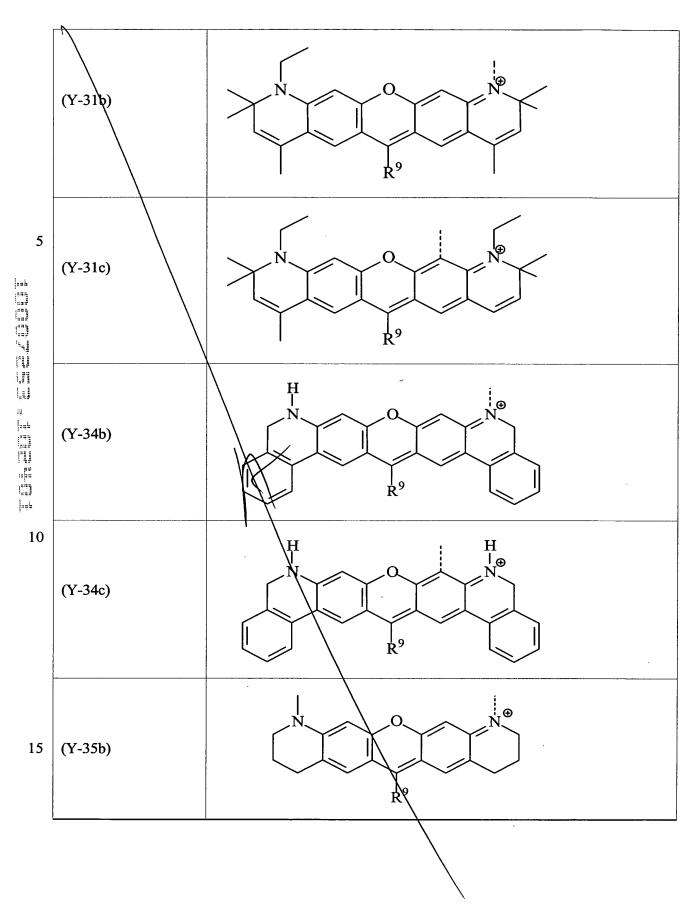
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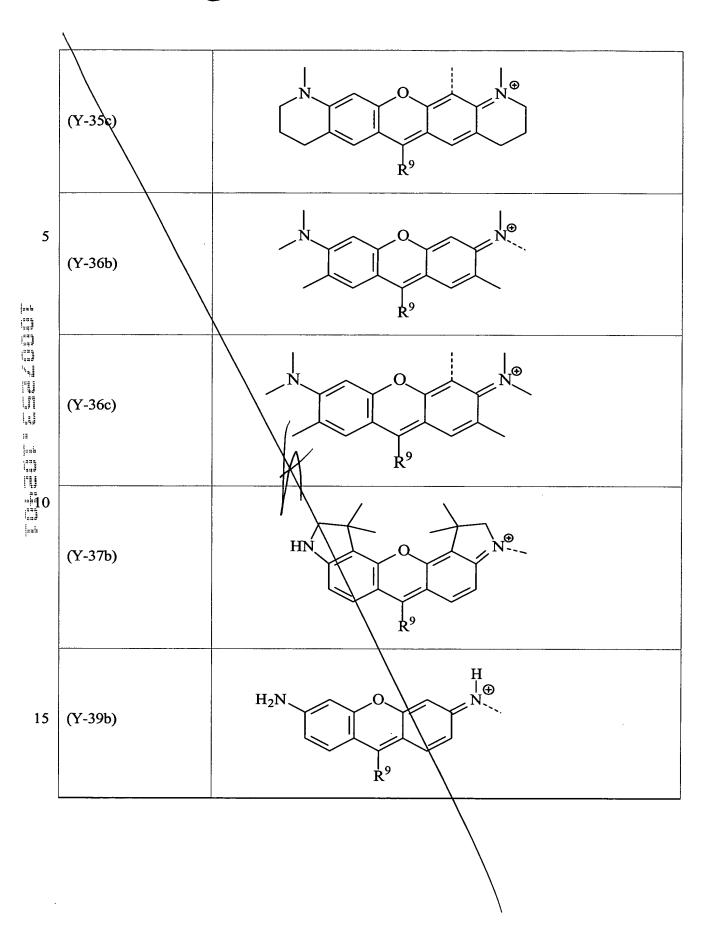
24. The rhodamine dye of Claim 18 in which Y<sup>1</sup> is selected from the group consisting

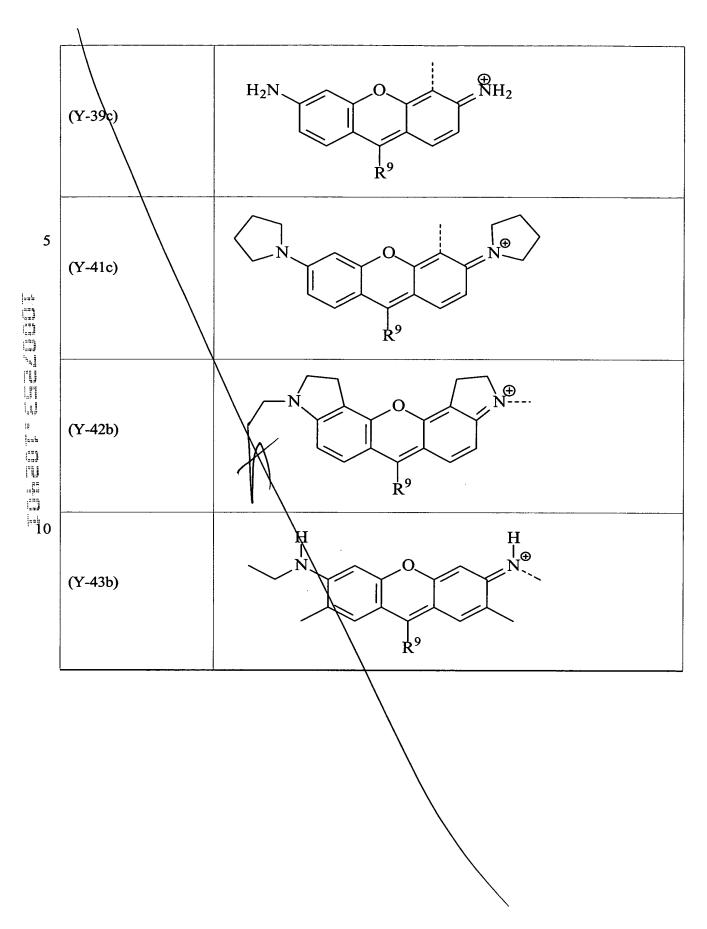


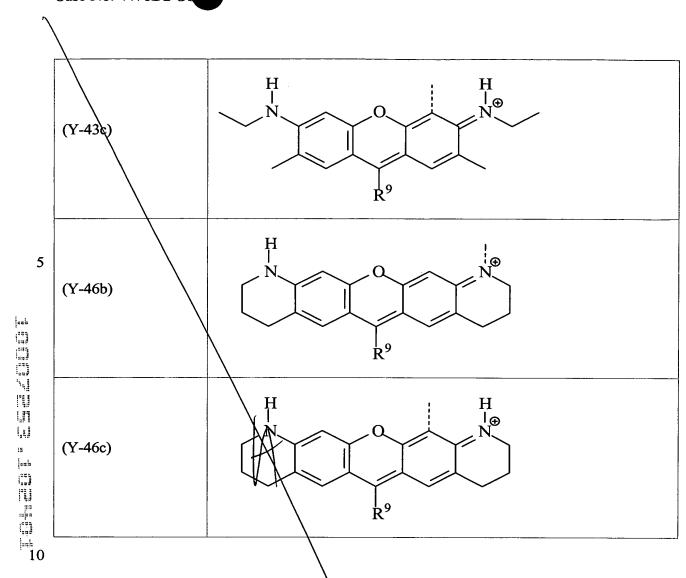












wherein R<sup>9</sup> and the dash at the nitrogen or C4 atom indicates the point of attachment of L.

25. The rhodamine dye of Claim 18 which has the structure:

or a salt thereof.

- 26. The rhodamine dye of Claim 25 in which Y<sup>1</sup> is selected from the group consisting of Y-1b, Y-2b, Y-3b, Y-4b, Y-1c, Y-2c, Y-3c and Y-4c.
- 27. The rhodamine dye of Claim 25 in which Y<sup>1</sup> is selected from the group consisting of Y-20b, Y-20c, Y-21b, Y-21c, Y-22b, Y-22c, Y-23b, Y-23c, Y-24b, Y-24c, Y-25b, Y-25c, Y-10 31b, Y-31c, Y-34b, Y-34c, Y-35b, Y-35c, Y-36b, Y-36c, Y-37b, Y-39b, Y-39c, Y-41c, Y-42b, Y-43b, Y-43c, Y-46b and Y-46c.
- An energy-transfer dye pair comprising a donor dye linked to an acceptor dye,
   wherein the donor dye or the acceptor dye is a compound according to Claim 1 and either or both
   of said donor and acceptor dyes include an optional linking morety.
  - 29. The dye pair of Claim 28 which has the structure:

or a salt thereof, wherein:

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electrophile;

R 1 is a covalent linkage formed upon reaction between a nucleophile and an

L" is a bond or a linker;

n is 1,  $\frac{\lambda}{2}$ , or 3; and

DD/AD is a donor dye or an acceptor dye which includes a linking moiety.

30. The dye pair of Claim 29 in which Y is selected from the group consisting of Y-1, Y-2, Y-3, Y-4, Y-20a, Y-21a, Y-22a, Y-23a, Y-24a, Y-25a, Y-31a, Y-34a, Y-35a, Y-36a, Y-39a, Y-41a, Y-42a, Y-43a, Y-44a, Y-46a.

31. The dye pair of Claim 29 in which L is a bond.

32. The dye pair of Claim 29 in which  $R^{41}$  has the formula  $-C(O)NR^{45}$ , where  $R^{45}$  is hydrogen or  $(C_1-C_6)$  alkyl.

- 33. The dye pair of Claim 29 in which  $Z^1$  is selected from the group consisting of  $(C_1-C_{12})$  alkyleno,  $(C_1-C_{12})$  alkano,  $(C_5-C_{10})$  aryldiyl and heteroaryldiyl, phenyldiyl, phena-1,4-diyl, naphthadiyl, naphtha-2,6-diyl, pyridindiyl and puriodiyl.
- 25 34. The dye pair of Claim 29 in which L" is  $-R^{43}-Z^3-C(O)-R^{44}-R^{45}$ , wherein  $R^{43}$  is  $(C_1-C_6)$  alkyldiyl, preferably  $(C_1-C_3)$  alkano, and is bonded to  $R^{42}$ , where  $R^{42}$  is O, S or NH;  $Z^3$  is 5-6 membered cyclic alkenyldiyl and heteroalkenyldiyl,  $(C_5-C_{14})$  aryldiyl and heteroaryldiyl;  $R^{44}$  is O, S or NH; and  $R^{45}$  is  $(C_1-C_6)$  alkyldiyl, preferably  $(C_1-C_3)$  alkano.

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- 35. The dye pair of Claim 29 in which DD/AD is a fluorescein dye in which the linking moiety is a reactive functional group and wherein L" is attached to the fluorescein dye at the xanthene C4 carbon.
- 36. The dye pair of Claim 29 which has the structure:

wherein, R<sup>50</sup> is a carboxyl, a salt, ester or activated ester thereof.

- 37. The dye pair of Claim 36 in which Y is selected from the group consisting of Y-1, Y-2, Y-3, Y-4, Y-20a, Y-21a, Y-22a, Y-23a, Y-24a, Y-25a, Y-31a, Y-34a, Y-35a, Y-36a, Y-39a, 15 Y-41a, Y-42a, Y-43a, Y-44a, Y-45a and Y-46a.
  - 38. The dye pair of Claim 28 which has the structure:

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$$\begin{array}{c|c}
 & L-R^{41}-L"-DD/AD \\
 & Y^1 & O \\
 & & OH \\
 & & S-Z
\end{array}$$

wherein:

R<sup>41</sup> is a covalent linkage formed upon reaction between a nucleophile and an electrophile;

L" is a bond or a linker;

n is 1, 2, or 3; and

DD/AD is a donor dye or an acceptor dye which includes a linking moiety.

- 39. The dye pair of Claim 38 in which Y<sup>1</sup> is selected from the group consisting of Y-1b, Y-2b, Y-3b, Y-4b, Y-1c, Y-2c, Y-2c, Y-4c, Y-20b, Y-20c, Y-21b, Y-21c, Y-22b, Y-22c, Y-23b, Y-23c, Y-24b, Y-24c, Y-25b, Y-25c, Y-31b, Y-31c, Y-34b, Y-34c, Y-35b, Y-35c, Y-36b, Y-36c, Y-37b, Y-39b, Y-39c, Y-41c, Y-42b, Y-43b, Y-43c, Y-46b and Y-46c.
  - 40. The dye pair of Claim 38 in which L is  $(C_1-C_6)$  alkyldiyl or  $(C_1-C_3)$  alkano.
- 41. The dye pair of Claim 38 in which  $R^{41}$  is an amide of the formula  $-C(O)NR^{45}$ , where  $R^{45}$  is hydrogen or  $(C_1-C_6)$  alkyl.
- 42. The dye pair of Claim 38 in which Z is selected from the group consisting of  $(C_1-C_{12})$  alkyl,  $(C_1-C_{12})$  alkanyl,  $(C_5-C_{10})$  aryl and heteroaryl, phenyl, naphth-1yl, naphth-2-yl, pyridyl and purinyl.
- 25 43. The dye pair of Claim 38 in which L" is  $-R^{43} Z^3 C(O) R^{44} R^{45}$ , wherein  $R^{43}$  is  $(C_1-C_6)$  alkyldiyl, preferably  $(C_1-C_3)$  alkano, and is bonded to  $R^{42}$ , where  $R^{42}$  is O, S or NH;  $Z^3$  is 5-6 membered cyclic alkenyldiyl and heteroalkenyldiyl,  $(C_5-C_{14})$  aryldiyl and heteroaryldiyl;  $R^{44}$  is

O, S or NH; and  $R^{45}$  is  $(C_1-C_6)$  alkyldiyl, preferably  $(C_1-C_3)$  alkano.

- 44. The dye pair of Claim 38 in which DD/AD is a fluorescein dye in which the linking moiety is a reactive group R<sub>x</sub> and wherein L" is attached to the fluorescein dye at the 5 xanthene C5 carbon.
  - 45. The dye pair of Claim 38 which has the structure:

10 wherein:

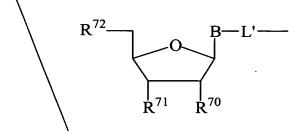
Y<sup>1</sup> is selected from the group consisting of Y-20b, Y-20c, Y-21b, Y-21c, Y-22b, Y-22c, Y-23b, Y-23c, Y-24b, Y-24c, Y-25b, Y-25c, Y-31b, Y-31c, Y-34b, Y-34c, Y-35b, Y-35c, Y-36b, Y-36c, Y-37b, Y-39b, Y-39c, Y-41c, Y-42b, Y-43b, Y-43c, Y-46b and Y-46c; and R<sup>50</sup> is a carboxyl, a salt, ester or activated ester thereof.

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46. The dye pair of Claim 45 in which Y<sup>1</sup> is selected from the group consisting of Y-1b, Y-2b, Y-3b, Y-4b, Y-1c, Y-2c, Y-3c, Y-4c, Y-20b, Y-20c, Y-21b, Y-21c, Y-22b, Y-22c, Y-23b, Y-23c, Y-24b, Y-24c, Y-25b, Y-25c, Y-31b, Y-31c, Y-34b, Y-34c, Y-35b, Y-35c, Y-36b, Y-36c, Y-37b, Y-39b, Y-39c, Y-41c, Y-42b, Y-43b, Y-43c, Y-46b and Y-46c.

A labeled nucleoside/tide or nucleoside/tide analog comprising the rhodamine dye of Claim 2 where Z has the form Z¹-L-R⁴6-L'-NUC, wherein:

R<sup>46</sup> is a linkage formed by reaction between an electrophile and a nucleophile; and -L'-NUC taken\together has the structure:



wherein:

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B is a nucleobase;

L' is  $(C_1-C_{20})$  alkyldiyl and heteroalkyldiyl,  $(C_1-C_{20})$  alkyleno and heteroalkyleno,  $(C_2-C_{20})$  alkyno and heteroalkyno or  $(C_2-C_{20})$  alkeno and heteroalkeno;

R<sub>70</sub> and R<sub>71</sub>, when taken alone, are each independently selected from the group consisting of hydrogen, hydroxyl and a moiety which blocks polymerase-mediated template-directed polymerization, or when taken together form a bond such that the illustrated 15 sugar is 2',3'-didehydroribose; and

R<sub>72</sub> is selected from the group consisting of hydroxyl, a phosphate ester having

ester analog, or a salt thereof.

48. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47 where Z has the form Z<sup>1</sup>-L-R<sup>41</sup>-L"-DD/AD-L<sup>3</sup>-R<sup>46</sup>-L'-NUC, or a salt thereof, wherein:

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 $\mathbb{R}^{41}$  is a covalent linkage formed upon reaction between a nucleophile and an electrophile;

L" is a bond or a linker;

DD/AD is a donor dye or an acceptor dye which includes a linking moiety; and.

L<sup>3</sup> is a bond or a linker.

49. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47 where Y has the form Y¹-R⁴¹-L"-DD/AD-L³-R⁴⁶-L'-NUC, or a salt thereof wherein:

$$Y^1$$
 is Y-1, Y-2, Y-3, or Y-4;

R<sup>41</sup> is a covalent linkage formed upon reaction between a nucleophile and an electrophile;

L" is a bond or a linker;

DD/AD is a donor dye or an acceptor dye which includes a linking moiety; and.

L<sup>3</sup> is a bond or a linker.

50. A labeled nucleoside/tide or nucleoside/tide analog of Claim 47 where Y has the form Y¹-R⁴¹-L"-DD/AD and Z has the form X¹-L-R⁴⁶-L'-NUC, or a salt thereof; wherein:

Y<sup>1</sup> is Y-1, Y-2, Y-3, or Y-4

R<sup>41</sup> is a covalent linkage formed upon reaction between a nucleophile and an

20 electrophile;

L" is a bond or a linker;

DD/AD is a donor dye or an acceptor dye which includes a linking moiety; and  $Z^1$  is  $(C_1-C_{12})$  alkyldiyl,  $(C_1-C_{12})$  alkyldiyl independently substituted with one or more of the same or different  $W^1$  groups,  $(C_5-C_{14})$  aryldiyl, and  $(C_5-C_{14})$  aryldiyl, heteroaryldiyl and 25 heteroaryldiyl independently substituted with one or more of the same or different  $W^2$  groups.

- 51. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47, 48, 49 or 50 which is enzymatically incorporatable.
- 30 52. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47, 48, 49 or 50 which is a terminator.

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54. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47 in which L' is selected from the group consisting of:

propargyl, where the terminal sp carbon is covalently attached to nucleobase B and the terminal methylene  $(sp^3)$  carbon is covalently attached to  $F_x$ ; and

 $-C = C - CH_2 - CH_2 - CH_2 - NR^{47} - R^{48}$ , where  $R^{47}$  is hydrogen or  $(C_1 - C_6)$  alkyl and  $R^{48}$  is  $-C(O) - (CH_2)_r$ ,  $-C(O) - CHR^{49}$ ,  $-C(O) - C = C - CH_2$  or  $-C(O) - \phi - (CH_2)_r$ , where each r is 10 independently an integer from 1 to 5 and  $\phi$  is  $C_6$  aryldiyl or heteroaryldiyl and  $R^{49}$  is hydrogen,

- $(C_1-C_6)$  alkyl or a side chain of an encoding or non-encoding amino acid, and where the terminal *sp* carbon is covalently attached to nucleobase B and the other terminal group is covalently attached to  $F_x$ .
- 55. The labeled nucleoside/tide or nucleoside/tide analog of Claim 48 or Claim 49 in which L<sup>3</sup> is a bond, R<sup>46</sup> the formula  $(C(O)-NHR^{51})$ , where R<sup>51</sup> is hydrogen or  $(C_1-C_6)$  alkyl.
- The labeled nucleoside tide or nucleoside/tide analog of Claim 47 in which nucleobase B is a purine, a 7-deazapurine an 8-aza,7-deazapurine, a pyrimidine, a normal nucleobase or a common analog of a normal nucleobase.
  - 57. The labeled nucleoside/tide or nucleoside/tide analog of Claim 47 or Claim 48 in which Y is selected from the group consisting of Y-1, Y-2, Y-3 and Y-4.
  - The labeled nucleoside/tide or nucleoside/tide analog of Claim 47 or Claim 48 in which Y is selected from the group consisting of Y-20a, Y-21a, Y-22a, Y-23a, Y-24a, Y-25a, Y-31a, Y-34a, Y-35a, Y-36a, Y-39a, Y-41a, Y-42a, Y-43a, Y-44a, Y-45a and Y-46a.
  - 59. The labeled nucleoside/tide or nucleoside/tide analog of Claim 49 or Claim 50 in 30 which Y¹ is selected from the group consisting of Y-1b, Y-2b, Y-3b, Y-4b, Y-1c, Y-2c, Y-3c and Y-4c.

62.

The labeled nucleoside/tide or nucleoside/tide analog of Claim 49 or Claim 50 in which Y1 is selected from the group consisting of Y-20b, Y-20c, Y-21b, Y-21c, Y-22b, Y-22c, Y-23b, Y-23c, Y-24b, Y-24c, Y-25b, Y-25c, Y-31b, Y-31c, Y-34b, Y-34c, Y-35b, Y-35c, Y-36b, Y-36c, Y-37b, Y-39b, Y-39c, Y-41c, Y-42b, Y-43b, Y-46b and Y-46c.

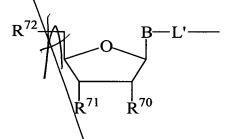
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A polynucleotide labeled with a rhodamine dye according to Claim 1 or an 61. energy-transfer dye pair according to Claim 28.

enzymatically extending a primer-target hybrid in the presence of a mixture of enzymaticallyextendable nucleotides capable of supporting continuous primer extension and a terminator, wherein said primer or said terminator is labeled with a rhodamine dye according to Claim 1 or an energy-transfer dye pair according to Claim 28.

A method of generating a labeled primer extension product, comprising the step of

63. The method of Claim 62 in which the terminator has the structure:



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wherein  $R_{70}$  and  $R_{71}$ , when taken alone, are each independently selected from the group consisting of hydrogen, halide, and any moiety which blocks polymerase-mediated template-directed polymerization.

64. The method of Claim 62 in which the terminator is a mixture of four different 25 terminators, one which terminates at a template A, one which terminates at a template G, one which terminates at a template C and one which terminates at a template T or U.

65. The method of Claim 62 in which each of the four different terminators is labeled with a different, spectrally-resolvable fluorophore.

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- 67. A method of detecting a rhodamine dye-antibody conjugate, in which said conjugate is a rhodamine dye-antibody conjugate according to Claim 66, comprising the steps of:
  - (a) binding the conjugate to a peptide or protein, and
  - (b) detecting the rhodamine dye-antibody conjugate bound to the peptide or protein.
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- 68. The method of Claim 67 in which the conjugate is bound to the peptide or protein in the presence of a second antibody specific for kinding said peptide or protein.
- 69. The method of Claim 68 in which the second antibody is bound to a solid bead or 15 particle.

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